AMENDMENTS TO THE SPECIFICATION

Please amend the paragraph beginning on page 5, line 5 as follows:

-- ~~Fig. 1. (a) Conservation~~ Figure 1 shows conservation of diphthamide domain and DT-resistant mutations in eukaryotic ~~EF-2s. (b) Nucleotide~~ EF-2s and nucleotide sequence mutations for the substitution of Arg for Gly 701 in *Pichia pastoris* EF-2. The underlined sequences are the site for the restriction enzyme *Sac* II that resulted from the nucleotide mutations. See SEQ ID NOS: 1-10. --

Please amend the paragraph beginning on page 17, line 18 as follows:

-- After gene optimization to reduce the AT content of the DNA sequence, secreted expression levels under the AOX1 promoter of 25- 30 mg/L can be obtained in bioreactors after 24-44 hours of induction. *Pichia pastoris* was sensitive to the toxic effects of cytosolic expressed diphtheria toxin A chain which ADP ribosylates elongation factor 2 (EF-2) leading to cessation of protein synthesis. Toxicity to expression of A-dmDT390-bisFv by the secretory route was indicated by a continuous fall in methanol consumption after induction. A mixed feed of glycerol and methanol was provided to the cells. Expression of the catalytic domain (A chain) of DT in the cytosol is lethal to *Pichia pastoris*. When cells bearing the construct A-dmDT390-BisFv (UCHT1) were induced by methanol to express the immunotoxin, nearly 50% were killed after 24 hours (Woo et al., 2002). In contrast, when the same immunotoxin was expressed in CHO cells that had been mutated to DT resistance, no toxic effect was observed (Liu, et al., 2000; Thompson, et al., 2001). In the cytosol of eukaryotes, the catalytic domain of DT catalyzes ADP ribosylation of elongation factor 2 (EF-2), leading to inhibition of protein synthesis and cell death (by protein starvation and or apoptosis, Van Ness et al., 1980; Houchins, 2000). The sensitivity of the eukaryotic EF-2 to ADP-ribosylation by these toxins lies in the structure of protein. EF-2 is a single polypeptide chain of about 850 amino acids and is composed of two domains. The N-terminal G domain is responsible for binding and hydrolysis of GTP that promotes translation, and the C-terminal R (or diphthamide) domain is thought to interact with

the ribosome (Kohno et al., 1986; Perentesis et al., 1992). The diphthamide domain (Fig.1[[a]]) contains a histidine residue in a region of 22 residues that are well conserved in the EF-2 of all eukaryotes. This conserved histidine is specifically modified post-translationally to the derivative, diphthamide, which is the unique target for ADP-ribosylation by DT (Van Ness et al., 1880). In *S. cerevisiae*, the conserved histidine can be mutated and substitutions with some other 2 amino acids yielded functional EF-2s that were resistant to ADP-ribosylation (Phan et al., 1993; Kimata and Kohno 1994). However, cells with EF-2 mutated at diphthamide grew more slowly than those expressing wild-type EF-2. In CHO cells, a single substitution of arginine for glycine, which is another well conserved residue located at the 3rd position to the C-terminal side of the diphthamide, also prevented the formation of diphthamide (Kohno & Uchida, 1987; Foley et al., 1992) and resulted in non-ADP-ribosylatable EF-2. This mutation had the same effect on EF-2 of *S. cerevisiae* (Kimata et al., 1993). In contrast to the mutation at diphthamide, the Gly to Arg mutation in EF-2 did not affect cell growth of CHO and *S. cerevisiae* (Foley et al., 1992; Kimata and Kohno 1994; Kimata et al., 1993). –

Please amend the paragraph beginning on page 36, line 17 as follows:

-- Transformation was performed with a partial DNA fragment containing the conserved region of the EF-2 gene and a mutation on amino acid 701. The partial sequence of *Pichia pastoris* EF-2 (positions 1717 to 2289, Fig. 3 (a) (b) (c) (d)) was mutated *in vitro* to change the amino acid 701 from glycine to arginine (Fig. 1[[b]]) and then co-transformed into the GS200 strain with the ARG4 gene fragment. More than 2000 Arg4 positive transformants were obtained and screened them for the EF-2 mutation by diagnostic PCR with primers mdb2EF-2 and 2253EF-2C. The mutation was not observed. –

Please amend the paragraph beginning on page 41, line 2 as follows:

-- After electroporation with the linearized pBLURA-Δ5'mutEF-2 DNA, Cells were spread onto plates containing synthetic complete medium for yeast minus uracil (K.D Medical, Maryland). Ura+ clones were then analyzed by "Colony PCR" for the presence the correct

mutations in the intact copy of EF-2. In this method, yeast cells from colonies were picked by tooth pickers and resuspended in 20ul of PCR mix. DNA released from the cells lysed by the first PCR step (94°C for 5 minutes) served as the template for PCR amplification. Five primers were used in the PCR detection procedures: primers 5'EF-2 and 3'EF-2C were described previously in section 4; EF-2 (1318) has the EF-2 sequence from position 1318 to 1341; primer wEF-2 is complementary to the positions 2100 to 2119, whereas primer mEF-2 has the sequence complementary the same positions but specific to the mutations The designed nucleotide mutations shown in Fig.1[[b]] created a new Sac II restriction enzyme site that was used to confirm the correct mutations in the genome. --